Original Research

Micronuclei Analysis in People Residing within 25 m of Radiation-Exposed Areas around Mobile Towers in Chennai, India: An Observational Study

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Abstract

Aim and Objectives: Mobile towers are known to emit a radiofrequency (RF) radiation continuously, which increases the health concern of living beings. However, still the biological effect of exposure to RF remains controversial. The present study was performed to evaluate the presence of micronuclei (MN) in the exfoliated buccal cells due to the genotoxic effect of cell phone tower radiation. **Materials and Methods:** This is an observational study that was conducted with randomly selected n = 108 subjects between 5 and 50 years of age from three school-based localities in Chennai, which were located within the radius of 10–25 m around the mobile towers; exfoliated buccal cells were collected. A smear was made using the collected exfoliated buccal cells and analyzed for the presence of MN. The smear was stained using giemsa stain to evaluate the same. The collected data were statistically analyzed using Pearson's chi-square test and Student's T-test. **Results:** The study results showed that 55.05% of the study population had the presence of MN in at least one field (a total of 10 fields examined), and 45.95% did not have MN in their exfoliated cells examined. The presence of MN was more seen in the 0–10 age groups, with preponderance of the female population ($P > 0.05$) being statistically insignificant. **Conclusion:** The presence of MN can be considered as a prerequisite for environmental genotoxicity and adverse health risks due to constant exposure to RF radiation.

Keywords: Buccal Cells, Micronuclei, Mobile Towers, Radiofrequency (RF) Radiation

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Introduction

Mobile phones have become indispensable devices in our daily life. According to the analytical report of the telecom regulatory authority of India (TRAI), the total number of wireless data connections increased from 424.02 million at the end of 2017 to 578.20 million at the end of 2018; accordingly, mobile towers are also increasing in number and are placed even in highly populated areas. Cell phone towers emit a radio frequency electromagnetic radiation (RF-EMR) of 1800 MHz.[1] These RF radiations are classified under nonionizing radiation.[2] Among Indian cities, Chennai is recorded as having the highest levels of RF radiation. In central Chennai, about 57.1% and in south Chennai about 25% of the mobile towers exceed the upper biological radiation limit of 0.5 mW/m^2.[3] The biological effect of exposure to RF remains controversial.[4] Long-term exposure to RF ranges from 100 KHz to 300 GHz; it generates electromagnetic fields, which can affect the living cell by damaging the DNA via thermal or nonthermal mechanisms.[4] DNA damage can lead to cell senescence, cell death, or malfunction. No scientific studies have yet provided conclusive evidence to prove that the use of cell

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phones is hazardous to human health.[3] Some studies have reported a causal relationship between exposure to RF electromagnetic fields and increased risk of acoustic neuroma, glioma, meningioma, infertility, deafness, and metabolic changes in brain tissue.[4] Some authors have confirmed the genotoxic effects of mobile phone radiations on oral mucosa and some have denied it.[1] Because of all this uncertainty in 2011, the World Health Organization classified RF-EMR on International Agency for Research on Cancer (IARC) scale as group 2B "possibly carcinogenic."[4] DNA damage caused by genotoxicants can be detected by several methods, including cytological observation of morphological changes such as MN, which are chromatin fragments resulting from chromosome breakage during abnormal mitosis.[4] MN are biomarkers of environmental genotoxicity and cancer risk.[4]

This study is designed to evaluate the presence of MN in exfoliated buccal mucosa cells by analyzing the samples from the radiation-exposed population.

Materials and Methods

Study design and study subjects

The study was conducted after getting approval from the ethical committee, institutional review board (IRB; ethical clearance number: IHEC/SDC/OPATH-1901/21/145). The study included 108 subjects in the age group of 5–50 years and who were randomly selected. The subjects were selected based on their residing locality and radius from the mobile towers. Three localities were selected, of which two were school-situated areas and the other was a public spot that was located within the radius of 10–25 m around the mobile towers. The subjects residing near mobile towers for at least a minimum of five years were randomly selected for the study. All subjects accepted the terms of informed consent; for subjects younger than the age of 18 years, consent was obtained from their parents and respective school heads, allowing the collection of samples from the oral buccal mucosa.

Sample collection and evaluation

A detailed questionnaire was prepared before sample collection to evaluate the lifestyle, dietary habits, previous history of medication, and locality of residence of the individual. The exfoliated cells from the buccal mucosa were collected from the study subjects using a moistened wooden spatula. The collected samples were spread evenly on a clean dry microscopic glass slide, and the slides were fixed immediately using isopropyl alcohol for a minimum of 30 min. The alcohol-fixed smears were stained using giemsa stain for it to be visible under a light microscope. The giemsa stain was poured onto the smear and retained for 10 min. Then, the stain was cleared by adding three to four drops of phosphate buffer (pH: 7.4) for 2 min. The slides were air dried and mounted. Each microscopic slide was observed in 10 different fields and evaluated for the presence of MN under 100× magnification with oil immersion by five observers.

Criteria for evaluation of MN

The criteria used for the evaluation of MN in the earlier mentioned study are as follows:

- Rounded smooth perimeter suggestive of a membrane.
- Less than one-third of the diameter of the associated nucleus, but large enough to discern the shape and color.
- Staining intensity similar to that of the nucleus.
- Texture similar to that of the nucleus.
- Same focal plane as follows.
- Absence of overlap with, or bridge to the nucleus.

Statistical analysis

Data were statistically analyzed using descriptive analysis to compare the frequencies between groups. The association between age and gender with the frequency of MN was analyzed using Pearson's chi-square test, and the gender was compared with MN using Student's T-test. The $P < 0.05$ was considered statistically significant. All analyses were conducted using the Statistical Package for Social Sciences (SPSS) software, IBM, version 20.0.

Results

A total of $n = 108$ subjects were microscopically evaluated in 10 different fields for the presence of MN from the exfoliated cells collected from the buccal mucosa. Out of $n = 108$, 78.90% were in the age group of 0–10 years; 7.33% were in the age group of 11–20 years; 5.50% were in the age group of 21–30 years; 4.82% were in the age group of 31–40 years; and 3.67% were in the age group of 41–50 years. Gender distribution among the study population shows that 52.29% were females and 47.71% were males. The demographic data of the study population are summarized in Table 1.

Analysis of MN shows that 55.05% of the study population had the presence of MN in at least one field (a total of 10 fields examined), and 45.95% did not have

Table 1: Percentage distribution of demographic data of the study population ($n = 108$)

Demographic data	%
Age (years)	
0–10	78.9
11–20	4.6
21–30	7.3
31–40	5.5
41–50	3.7
Gender	
Males	52.3
Females	47.7

MN in their exfoliated cells examined [Figure 1]. The presence of MN in the exfoliated buccal cells is shown in photomicrography [Figure 2].

The association between the presence and absence of MN among different age groups showed that 47.1% of the subjects in the age group of 0–10 years had micronucleated cells; 3.67% of the subjects in the age group of 21–30 years had micronucleated cells; 1.83% of the subjects in the age group of 11–20 years had micronucleated cells; and 0.92% of the subjects in both the age groups of 31–40 years and 41–50 years had micronucleated cells. Statistical results show that there is no significant association between the presence and absence of MN among different age groups $P = 0.159$ ($P > 0.05$, statistically insignificant) [Figure 3].

The presence/absence of MN in association with gender showed that 29.36% of males had MN and 18.35% had no MN. Overall, 25.69% of females had MN and 26.91% had no MN. The chi-square statistical results showed a P value of 0.193 ($P > 0.05$, statistically insignificant) [Figure 4].

The Student's T-test on comparison with gender and MN showed P value of 0.564 ($P > 0.05$, statistically insignificant). The P values are further listed in Table 2.

Discussion

MN are extranuclear bodies that contain altered chromosome sections or potentially entire chromosomes that were not consolidated into the nucleus after cell division.[5] The MN represent a biological phenomenon in themselves, providing us a clue to understand the mechanism of nucleus reconstruction and the malignant phenotype of cells.[6] A variety of genotoxic agents may induce MN formation, and RF radiation is one such factor causing MN formation. The RF radiation is called a nonionizing radiation, because it potentially heats tissues but the intensity and energy is sufficient to cause ionization.[7] These nonthermal exposure levels have resulted in biological effects in humans, animals, and cells, leading to cell death, genomic instability, or cancer development. Because of the expanding utilization of wireless technology, ecological introduction to RF radiation has dynamically increased.[7] We have performed this study to evaluate the presence of MN in their exfoliated buccal mucosa cells and to correlate it with the genotoxic effect due to RF radiation effect at a regional level in Chennai.

The results of our study showed that the presence of MN was noted more in the younger age group of 0–20 years (49.54%) than the adults of 21–50 years (5.51%). Overall, 78.9% of our study population was younger than 10 years

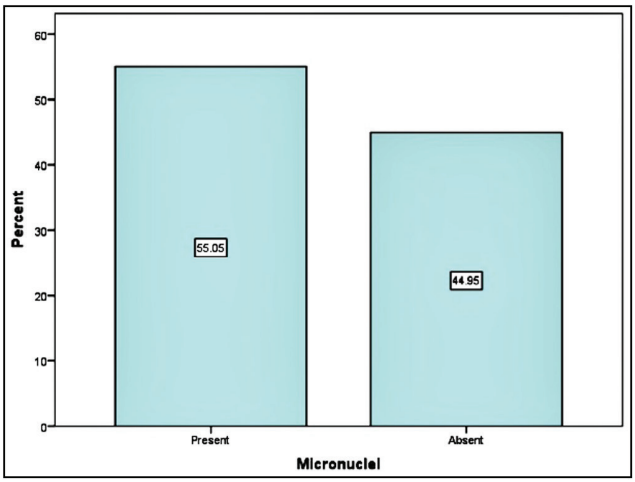

Figure 1: Bar chart depicting the percentage distribution of the presence and absence of MN from the exfoliated buccal mucosa cells of the study subjects ($n = 108$)

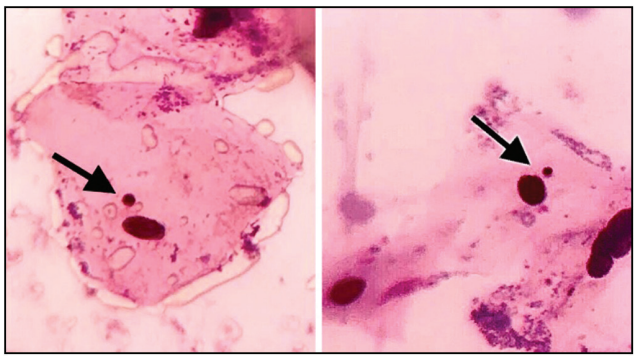

Figure 2: The photomicrograph (100× magnification) showing the presence of MN from the exfoliated buccal mucosa cells. The arrow points toward the MN

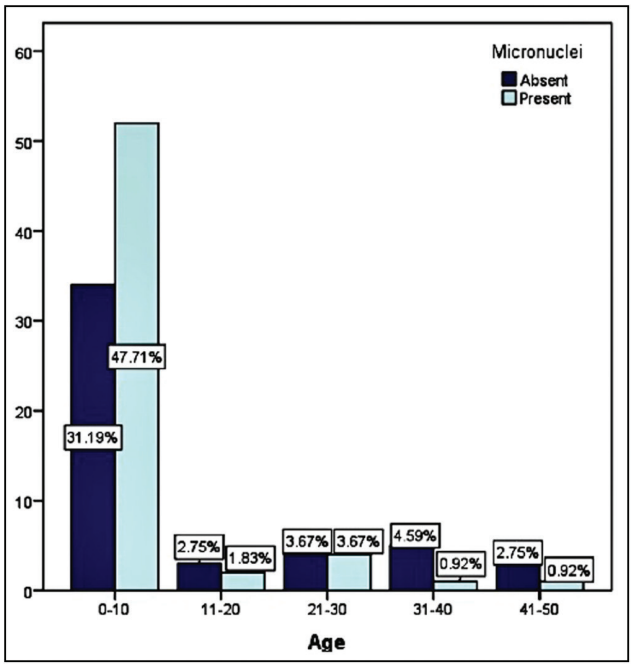

Figure 3: Bar chart depicting the percentage distribution of the presence/absence of MN from the exfoliated buccal mucosa cells among the different age groups of the study subjects ($n = 108$). The chi-square results showed P value = 0.159 ($P > 0.05$)

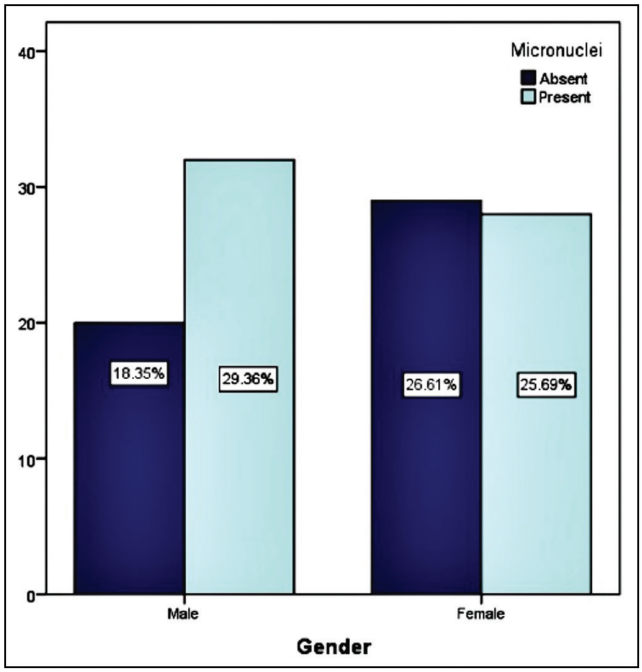

Figure 4: Bar chart depicting the percentage distribution of the presence/absence of MN from the exfoliated buccal mucosa cells among the gender groups of the study subjects ($n = 108$). The chi-square results showed P value = 0.193 ($P > 0.05$)

Table 2: *P* values of statistical analysis using Pearson's chi-square test and Student's T-test

Statistical analysis	Variables	*P* value
Pearson's chi-square test	Micronuclei × Age	0.159
	Micronuclei × Gender	0.193
Student's T-test	Micronuclei × Gender	0.564

of age because of the concern that the younger population will be most likely exposed for an entire lifetime, during which the illness may manifest at a later age. No similar studies have estimated the presence of DNA damage according to age. However, a few studies estimated the exposure and absorption of RF radiation in accordance with age and its other adverse health effects. Gandhi, *et al.*[8] stated that deeper penetration of absorbed energy was seen in children, which is 53% higher than adults. Wiart, *et al.*[9] noticed that the absorption rate of radiofrequency electromagnetic radiation (RF-EMR) of brain tissues of children is about two times higher than adults. Peyman, *et al.*[10] stated that children's absorption is 50%–100% higher than adults. Previous literature emphasized the adverse health effects caused by irradiation over a longer period of life. Their study was conducted on a population whose living ambience was situated <80 m from a mobile phone base station; this showed that RF radiation induced DNA damage, a lowering of antioxidant levels, and a higher frequency of MN in peripheral blood lymphocytes, sleep disturbances, burned-out syndromes, and depression.[11] This is in accordance with our study, where age plays a pivotal role in the emergence of DNA damage (micronucleation). This is because the bodies of the children are in a continuous state of development and growth until adulthood; the more the presence of immature cells, the more the RF radiation absorption rate in children as per the exposure rate. So, the rapid technological advancements and their increasing usage in day-to-day life lead to more physiological and psychological disturbances in living beings regardless of their age.[7]

Consequently, the female population in this study was observed to have more MN (29.36%) in their exfoliated buccal mucosa cells than the male population (25.69%). Gandhi, *et al.*[12] identified that damage frequency and DNA migration length levels were higher in females but did not reach significant levels; also, significantly elevated frequencies of micronucleated cells (3.76-fold) were observed. This can be attributed to more inhibition of cell proliferation exhibited in males.[12] In contrast, Zothansiama, *et al.*[11] noticed that MN frequency showed no significant variations between the ages and genders within the exposed group. Interestingly, limited research was done on animals to evaluate the presence of adverse health effects due to the exposure to RF radiation. It was found that physiological differences between females and males do make an impact on the permeability/absorption of the RF radiation.[13,14]

Overall, our study showed that 55.05% of the study population had the presence of micronucleated cells. There were also differences in the number of MN among people exposed to the same environment. Previous literature supports the presence of MN due to RF radiation exposure. Banerjee, *et al.*[1] found that the number of micronucleated cells in exfoliated buccal cells was found to be increased in high mobile phone users due to increased exposure to mobile phone RF radiation. Daroit, *et al.*[2] found that there is a significant increase in MN in individuals who use their mobile phones for a longer duration. In their study, Yadav and Sharma[15] found that the frequency of micronucleated cells was more in mobile phone users. Gandhi and Prabhjot[16] found a positive correlation between RF radiation from mobile phones and the presence of MN in the exfoliated cells. All these studies have emphasized the effects of RF radiation but those that are emitted from the mobile phones and not their stations. Unfortunately, there is no conclusive evidence stating that mobile towers do emit radiation directly onto us like our mobile phones, laptops, and other electronic devices that emit RF radiation continuously.[17] There is still a dilemma that living, working, or going to school near a cell phone tower might increase the risk of cancer or other health problems. At this time, there is not much evidence to support the mobile tower RF emission and its positive occurrence of adverse health effects. However, when a mobile phone that emits approximately

800 MHz is known to cause serious health issues, why not consider the mobile towers that emit approximately 900–1800 MHz, even though the emission is not directly onto us?[18] Therefore, the presence of MN can be considered as a promising prerequisite for environmental genotoxicity and adverse health risks due to constant exposure to RF radiation.

The authors acknowledge a few limitations in this study. The study subjects were not evenly distributed among diverse age groups and the subjects were not evaluated for the amount of exposure to the RF radiation in workplaces as well as residing places. The amount of exposure can be subjective depending on the usage of gadgets and depending on the amount of duration of exposure, whether direct or indirect. Taking all these into consideration, we cannot positively conclude that the presence of MN is solely because of the constant or intermittent exposure from the mobile tower RF radiation but it can be considered in the future to decrease the access to areas with mobile towers to possibly eliminate adverse health effects, if any. This is a preliminary analysis that considered the study variables' age and gender as their only independent variables. To substantiate the results of this research, further large-scale longitudinal studies will be needed.

Future research scope

This research information would help raise knowledge and awareness among the general population living near cell towers regarding the potentially harmful radiation emitted from the towers and its associated health ill effects, if any. As a consequence, the general public will finally take measures to limit the exposure.

Conclusion

Our study revealed that 55.05% of the study population residing within 25 m exhibited MN in their exfoliated buccal cells; among these, 52.29% were females. This evidence shows that long-term exposure to radiation from mobile towers, combined with other possible factors, does induce MN formation, especially in the younger population, which may ultimately end up in chronic illness in later stages of life.

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Conflicts of interest

There are no conflicts of interest.

Author contributions

All of the authors contributed to the design, data analysis, results analysis, article writing, and final approval of the publication.

Ethical policy and institutional review board statement

The Institutional Review Board granted ethical approval for this study (ethical clearance number: IHEC/SDC/OPATH-1901/21/145).

Declaration of patient consent

Not applicable.

Data availability statement

The datasets generated during and/or analyzed during the current study are available from Saveetha Dental College, Saveetha University (SIMATS).

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